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Specific allergy to *Penaeus monodon* (seawater shrimp) or *Macrobrachium rosenbergii* (freshwater shrimp) in shrimp-allergic children

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Clinical and Experimental Allergy

Summary

Background Allergy to specific shrimp species has not been studied systematically by oral challenges. A comparison of allergy to different shrimp species, especially seawater or freshwater varieties treatment, would be useful in testing shrimp-allergic subjects.

Objective The aim of the study was to identify cases of specific allergy to seawater shrimp, *Penaeus monodon* (*Pm*), or freshwater shrimp, *Macrobrachium rosenbergii* (*Mr*), among shrimp-allergic children. Comparisons of skin tests using commercial and crude shrimp extracts plus the prick-to-prick (PTP) method were investigated.

Methods Sixty-eight children with a history of shrimp allergy and skin tests positive to shrimp were orally challenged to both shrimp species. Reactivity to skin prick tests using extracts of *Pm* (*Pm*SPT), *Mr* (*Mr*SPT), commercial shrimp (ComSPT), and PTP tests (*Pm*PTP, *Mr*PTP) was compared.

Results Food challenges identified specific allergy to *Pm* and *Mr* in 17.65% and 23.53% of the subjects, respectively. Positive and negative challenges to both shrimp species were found in 47.06% and 11.76% of the subjects, respectively. Correlations between the mean weal diameter (MWD) from ComSPT–*Pm*SPT, ComSPT–*Pm*PTP, ComSPT–*Mr*PTP, *Pm*SPT–*Pm*PTP and *Mr*SPT–*Mr*PTP, but not ComSPT–*Mr*SPT, were observed. The MWD from *Pm*SPT and *Pm*PTP were significantly larger in patients with positive than negative challenges to *P. monodon* ($P < 0.05$). There was a trend that MWD from *Mr*SPT were larger in patients with positive than negative challenges to *M. rosenbergii* ($P = 0.058$). In the *Pm* allergy group, *Pm*SPT with an MWD of 30 mm provided 80% predictive probability for positive challenges. *Pm*PTP and ComSPT with an MWD of 22.5 and 20 mm provided 95% predictive probability, respectively. In the *Mr* allergy group, *Mr*SPT with an MWD of 30 mm provided 95% predictive probability.

Conclusion Specific allergy to *Pm* or *Mr* was confirmed by food challenges. SPT using crude extracts and the PTP test are useful tools for screening shrimp sensitization before a food challenge. The predictive probability of SPT is helpful where a food challenge is not feasible.

Keywords food allergy, food challenge, *Macrobrachium rosenbergii*, *Penaeus monodon*, predictive probability, prick-to-prick test, shrimp allergy, shrimp extract, skin prick test
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Introduction

Shellfish are recognized as a common cause of food hypersensitivity and as the leading cause of food-induced anaphylaxis [1, 2]. Among shellfish allergies, shrimp is the most frequent culprit [2, 3]. Symptoms of shrimp allergy develop in multiple organs such as the skin (52–90%), respiratory (42%), gastrointestinal (GI, 35%), and shock (10%) [4]. The major shrimp allergen, tropomyosin, has been identified as a pan-allergen commonly found in other invertebrates such as lobster, crab, mollusk, squid, cockroach, and house dust mite [5–8]. Cross-reactivity between shrimp and other crustaceans has been demonstrated [9]. However, there was also a report, that suggested isolated allergy to specific shrimp species [10].

Two popular shrimp species, *Penaeus monodon* (black tiger shrimp) and *Macrobrachium rosenbergii* (giant fresh water prawn), are harvested in Thailand. *P. monodon*, which is cultivated in saltwater, is the most widely distributed and marketed shrimp in the world. *M. rosenbergii*, which is cultivated in freshwater, is increasingly being recognized in the world market due to its texture and palatability. Surprisingly, there is no report of *M. rosenbergii* allergy in any medical literature. From the anaphylaxis study at the Siriraj hospital, Thailand, there were subpopulations of shrimp-allergic patients who developed anaphylaxis to freshwater shrimp but could tolerate seawater shrimp or vice versa [2].

The objective of this study was to identify cases of *P. monodon* or *M. rosenbergii*-specific allergy in shrimp-allergic children. The importance of skin tests using raw *P. monodon* or *M. rosenbergii* extracts and the prick-to-prick (PTP) method using cooked *P. monodon* or *M. rosenbergii* compared with commercial shrimp extract was also investigated.

Materials and methods

Subjects

The study was approved by the Institutional Review Boards, Siriraj Hospital. Informed consent was obtained from the patients (or parents if patients were <18 years of age). Patients $\geqslant 5$ years of age who came to the paediatric allergy clinic, Siriraj Hospital, Thailand, from 1 June 2005 to 31 December 2006, with a history of shrimp allergy were asked to participate in this study. Patients with a history of a severe anaphylactic reaction from seafood, pregnancy, underlying diseases such as cardiovascular (CVS), hepatobiliary, and renal diseases were excluded. Pregnancy tests were conducted in order to exclude pregnant subjects. None of the patients was on systemic corticosteroid or β-blocking agents. Patients with allergic diseases were asymptomatic on the days of skin tests and food challenges. Patients with asthma were asymptomatic and had not used a bronchodilator in the past 7 days before the skin tests and food challenges. The peak expiratory flow was $>70\%$ of the predicted value on the days of the skin tests and food challenges.

Skin-test procedure [11]

The skin prick tests (SPT) using raw *P. monodon* and *M. rosenbergii* extracts (*Pm*SPT and *Mr*SPT) as well as the PTP method using cooked *P. monodon* and *M. rosenbergii* (*Pm*PTP and *Mr*PTP, pricking the food and then pricking the skin of the patients) were performed with 10 mg/mL of histamine phosphate and glycerinated saline as positive and negative controls. All skin tests were performed using straight surgical needles on the back of the patients. The shells and heads of the specific shrimps were removed before lyophilization or cooking. The meat was chopped and frozen at $-70\,^{\circ}$C for 24 h before the lyophilization process. The *P. monodon* and *M. rosenbergii* extracts were freshly prepared by making a dilution of 1 : 10 (wt/volume) of raw lyophilized shrimp in Coca's solution. The mixture was stirred at room temperature for 1 h before centrifugation at 21 652 g. The supernatant was sterile filtered before use. This concentration proved to be a non-irritating concentration in 10 non-atopic subjects (data not shown). SPT to commercial extracts (Center Laboratory, Port Washington, NY) of shrimp (made from raw *Penaeus aztecus*, ComSPT), *Dermatophagoides pteronyssinus (Dp)*; *Dermatophagoides farinae (Df),* and *Periplaneta americana* (American cockroach, *Pa*) were also performed. Antihistamines were discontinued for $\geqslant 7$ days before skin testing. The size of weal and flare reactions was recorded in millimetres (mm). The mean of the largest and midpoint orthogonal diameters was designated as the mean weal diameter (MWD), and considered to be positive if it was $\geqslant 3$ mm compared with the negative control.

Food-challenged procedure [11, 12]

Food challenges to *P. monodon and M. rosenbergii* were performed in patients with skin tests positive to ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP, or *Mr*PTP. Shrimp was eliminated from the diet for 2 weeks before a challenge. The food-challenge protocol contained three steps of challenges with multiple doses in two of the steps. Each step was 15 min apart. The provocation dose (PD) schedule of raw lyophilized shrimp in capsules was as follows: 500 mg, 1, 2, 4, and 8 g at a 15-min interval. The cumulative PD was 15.5 g. Young patients who could not swallow capsules were allowed to skip this process. To identify oral-mucosal reactions, 2 g of cooked shrimp was wiped on the inner lips and placed in the mouth without chewing and spat out after 5 min. Lips' swelling/itching or throat itching were recorded as positive oral-mucosal reactions. Fifteen minutes later, open feeding to cooked shrimp was initiated. The PD schedule of cooked shrimp was as follows: 1, 2, 4, 8, 16, and 32 g at a 15 min interval. The cumulative PD was 63 g. Positive responses to any three types of challenge were considered to be a positive challenge. Anaphylaxis was diagnosed by the recent criteria [13]. Vital signs as well as patient's symptoms and signs were recorded every 15 min. Emergency resuscitation equipments and drugs were available in case of an emergency. Challenge to the other species of shrimp was conducted 2–4 weeks after the first challenge.

Data collection and analysis

Data were expressed as individual values or the mean $\pm$ SD for groups. Median and range were used for non-normally distributed data. Spearman's ρ correlations

were used to compare MWD from the different methods of skin tests in all patients. Comparisons of MWD from skin tests between groups of positive and negative challenges were made using the Mann–Whitney *U*-test. Differences between groups were considered to be significant at a *P*-value of $\leqslant 0.05$. The logistic regression analysis was used to predict probabilities of the outcome of food challenges by MWD of different skin tests.

Results

Demographic data

Seventy-two patients who had a history of shrimp allergy and a positive skin test to either ComSPT, *Pm*SPT, *Pm*PTP, *Mr*SPT, or *Mr*PTP were recruited. Four patients developed anaphylaxis on the first challenge and were excluded from the study. For three patients who developed anaphylaxis to *P. monodon*, two developed at the cumulative dose of 3.5 g of lyophilized raw shrimp while the other developed at 7.5 g of lyophilized raw shrimp. The MWD of skin tests reactions of these three patients to ComSPT, *Pm*SPT, and *Pm*PTP were in the fourth quartile of those reactions in patients with positive challenges to *P. monodon*. One patient developed anaphylaxis to *M. rosenbergii* at 15 min after 2 g of cooked shrimp was wiped on the inner lips and placed in the mouth. She did not take the raw lyophilized shrimp in capsule form. The MWD of skin test reaction of this patient to ComSPT was in the third quartile and that of *Mr*SPT and *Mr*PTP were in the fourth quartile of those reactions in patients with positive challenges to *M. rosenbergii*.

Demographic data, clinical history, results of skin tests, cumulative PD of shrimp, and symptoms upon challenges are shown in Tables 1–4. Overall, the 68 patients who completed both challenges were divided into four groups: group I patients, who had positive challenges to *P. monodon* only ($n = 12$, 17.65%), group II patients, who had positive challenges to *M. rosenbergii* only ($n = 16$, 23.53%), group III patients, who had positive challenges to both shrimp species ($n = 32$, 47.06%), and group IV patients, who had negative challenges to both shrimp species ($n = 8$, 11.76%). Sixty-five of 68 patients reported that they had allergic reactions to both *M. rosenbergii* and *P. monodon*. Three patients reported that they had allergic reactions to *P. monodon* only. These three patients were in the group I upon challenges.

Underlying allergic diseases and clinical history of shrimp allergy

All patients had underlying allergic diseases, with 94.12% respiratory allergies (44.12% asthma, 72.06% allergic rhinitis, and 14.71% allergic rhinoconjunctivitis), 13.24% urticaria, 7.35% atopic dermatitis, and 2.94% vernal keratoconjunctivitis. Clinical histories of shrimp allergy were reported mainly in the skin–mucosal system (92.65%) such as lip swelling/itching, urticaria, flushing, pruritus, and angiooedema, followed by the respiratory (29.41%), GI (14.71%), and cardiovascular system (CVS, 1.47%).

Results of food challenges

The results of food challenges are shown in Tables 1–3. All the patients with positive challenges developed symptoms during the challenge or immediately after test completion. The lowest cumulative PD of both *P. monodon* and

Table 1. Demographic data, clinical history, skin test results, cumulative provocation doses of *P. monodon* and symptoms upon challenges in group I patients who had positive food challenges to *P. monodon* but negative food challenges to *M. rosenbergii*

No.	Sex	Age (years)	History of reactions to shrimp	Mean weal diameter from skin tests (mm)					*P. monodon* cumulative PD			Symptoms upon challenges
				ComSPT	*Pm*SPT	*Pm*PTP	*Mr*SPT	*Mr*PTP	Raw shrimp (g)	Oral-mucosal reaction	Cooked shrimp (g)	
1	M	12	LSI, NV	3	8	7.5	15	10	15.5	No	7	TI
2	M	14	LSI, U	6	20	15	12.5	17.5	ND	No	31	LSI, U
3	M	15	AG, R	5	21	13	15	12.5	ND	No	31	TI
4	M	10	LI, R, SP	8	22.5	17.5	21	25	7.5	ND	ND	W, R, CP, NV, A
5	M	15	F, P	3	9.5	8	7.5	6	15.5	ND	ND	F, P
6	M	13	LSI, U, W	3	5.5	5	6	4.5	15.5	No	31	TI, CP
7	M	12	U, R	14	17.5	8	20	11.5	15.5	ND	ND	U
8	M	9	LSI, U	4	6.5	9	4	11.5	3.5	Yes	31	LSI, U
9	M	8	LSI, NV	9	11	3	8.5	8.5	15.5	Yes	3	LSI, AP, A
10	M	11	LSI, R	4	9.5	4.5	0	4	15.5	Yes	15	LSI, R
11	F	6	LI, U	3	11.5	15	3	7	ND	Yes	31	LSI, U, AG
12	M	15	LI, F, U	4	12.5	12.5	8	12.5	15.5	No	31	F

A, anaphylaxis; AG, angioedema; AP, abdominal pain; ComSPT, skin prick test using commercial shrimp extract; CP, chest pain; F, flushing; LI, lip itching; LSI, lip swelling/itching; *Mr, M. rosenbergii*; ND, not done; NV, nausea/vomiting; P, skin pruritus without rash; PD, provocative dose; *Pm*, *P. monodon*; PTP, prick-to-prick tests; R, rhinitis; SP, substernal pain; SPT, skin prick tests; TI, throat itching; U, urticaria; W, wheezing; F, female; M, male.

Table 2. Demographic data, clinical history, skin test results, cumulative provocation doses of *M. rosenbergii* and symptoms upon challenges in group II patients who had positive food challenges to *M. rosenbergii* but negative food challenges to *P. monodon*

No.	Sex	Age (years)	History of reactions to shrimp	Mean weal diameter from skin tests (mm)					*M. rosenbergii* cumulative PD			Symptoms upon challenges
				ComSPT	*Pm*SPT	*Pm*PTP	*Mr*SPT	*Mr*PTP	Raw shrimp (g)	Oral-mucosal reaction	Cooked shrimp (g)	
1	M	10	LI, U	5.5	8.5	8	11.5	5.5	15.5	Yes	3	LI, U
2	M	10	LSI, U	3.5	4.5	6	12	17.5	15.5	Yes	ND	LSI, U
3	F	9	LI, U	3	4.5	4	8	5.5	15.5	No	63	LSI, U
4	F	14	U	0	2.5	3	3	3	15.5	Yes	ND	LI, U
5	F	9	LSI, U	3	3	5.5	6.5	4	15.5	Yes	7	LSI, U
6	M	6	LSI, U, AG	8.5	15.5	9	8	6.5	15.5	Yes	ND	LSI, AG
7	M	14	U, W, SP	3	7.5	12	13	10	ND	No	31	LI, R
8	M	8	LSI, U	4	11.5	4	11	10	15.5	Yes	31	LSI
9	M	9	LSI	3	6.5	4	17.5	16.5	15.5	No	1	LSI, U, AP, NV, A
10	M	8	LSI	7.5	25	7	18.5	18.5	15.5	Yes	ND	LSI
11	F	14	TI	0	7	4	5	9	15.5	Yes	31	LSI
12	F	5	LSI	6	7.5	8	13.5	6.5	ND	Yes	ND	LSI, U
13	M	14	LSI, U, AG	3	4.5	5	3	3.5	15.5	Yes	ND	LSI, U, AG
14	M	11	LSI, U	3	10	5	17.5	7.5	15.5	Yes	ND	LSI, U, TI
15	M	9	LI, R, Di	3.5	24	17	18.5	9	15.5	Yes	ND	LSI, U
16	F	10	LI, R, Di	3.3	4	4	5.5	7.5	15.5	Yes	ND	LSI, U

A, anaphylaxis; AG, angioedema; AP, abdominal pain; ComSPT, skin prick test using commercial shrimp extract; Di, diarrhea; F, flushing; LI, lip itching; LSI, lip swelling/itching; *Mr*, *M. rosenbergii*; ND, not done; NV, nausea/vomiting; P, skin pruritus without rash; *Pm*, *P. monodon*; PTP, prick-to-prick tests; R, rhinitis; SP, substernal pain; SPT, skin prick tests; TI, throat itching; U, urticaria; W, wheezing; F, female; M, male.

M. rosenbergii in raw forms were 3.5 g and that for cooked forms were 1 g. Positive reactions to raw *P. monodon* and *M. rosenbergii* were found in 6/41 (14.63%) and 2/46 (4.35%) of the challenges, respectively ($P = 0.09$). Oral-mucosal reactions to *P. monodon* and *M rosenbergii* were found in 24/38 (63.16%) and 35/46 (76.09%) of the challenges, respectively. Positive reactions to cooked *P. monodon* and *M. rosenbergii* were found in 25/25 (100%) and 23/23 (100%) of the challenges, respectively. Patient no. 8 of group I refused to swallow a capsule after he took 3.5 g of *P. monodon* capsules. Nine patients in group II, and 13 patients (challenged with *P. monodon)* and 14 patients (challenged with *M. rosenbergii)* in group III, who had significant oral-mucosal reactions, refused to ingest cooked shrimp.

The most common symptom upon challenges was found in the skin-mucosal system (95.65%) such as lip swelling/itching, urticaria, flushing, skin pruritus, throat itching, eye itching, and angioedema, followed by respiratory (23.91%), GI (16.3%), and CVS (3.26%). Anaphylaxis was found in 11.95% of the challenges. Anaphylaxis to *P. monodon* was found in two and three patients who ingested raw and cooked shrimp, respectively. Anaphylaxis to *M. rosenbergii* was found in three patients who ingested cooked shrimp and three patients who were on the step of testing oral-mucosal reactions. None of the patients who ingested raw *M. rosenbergii* developed anaphylaxis. Symptoms from *P. monodon* or *M. rosenbergii* were not significantly different (data not shown). In groups I–III, who developed symptoms upon challenges, there was a good spread of symptoms in terms of organ involvements. It might be difficult to compare the severity of each group as some patients refused to carry out one of the challenge steps and therefore did not receive a full challenged dose to develop full symptoms.

Results of skin tests

All patients had at least one positive skin test to shrimp. Most patients had positive reactions to all skin tests including ComSPT, *Pm*SPT, *Mr*SPT, *Pm*PTP, and *Mr*PTP. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of different skin tests are shown in Table 5. The NPV was not applicable for *Mr*PTP because no patient had negative *Mr*PTP. Correlations of MWD from different skin tests in all patients were tested (Fig. 1). There were fair correlations between MWD from ComSPT–*Pm*SPT (Fig. 1a), ComSPT–*Pm*PTP (Fig. 1b), and ComSPT–*Mr*PTP (Fig. 1d). However, there was no correlation between MWD from ComSPT–*Mr*SPT (Fig. 1c). A moderate correlation between MWD from *Pm*SPT–*Pm*PTP (Fig. 1e) and a fair correlation between MWD from *Mr*SPT–*Mr*PTP (Fig. 1f) were observed.

MWD from different skin tests were further compared in groups of patients with positive or negative challenges to *P. monodon* or *M. rosenbergii* (Fig. 2). Overall, the median MWD from all the skin tests were larger in the groups with positive (groups I and III) than negative challenges

Table 3. Demographic data, clinical history, skin test results, cumulative provocation doses of *P. monodon* and *M. rosenbergii* and symptoms upon challenges in group III patients who had positive food challenges to both *P. monodon* and *M. rosenbergii*

No.	Sex	Age (years)	History of reactions to shrimp	Mean weal diameter from skin tests (mm)					*P. monodon* cumulative PD			Symptoms upon challenges with *Pm*	*M. rosenbergii* cumulative PD			Symptoms upon challenges with *Mr*
				ComSPT	*Pm*SPT	*Pm*PTP	*Mr*SPT	*Mr*PTP	Raw shrimp (g)	Oral-mucosal reaction	Cooked shrimp (g)		Raw shrimp (g)	Oral-mucosal reaction	Cooked shrimp (g)	
1	M	10	LI, U, R, CP	3	6	3	10	7.5	15.5	No	3	LI, CP, R	ND	No	15	LI, U, R
2	M	13	LSI, U, R	7.5	8.5	15	9.5	11	15.5	No	31	LI, U, R, EI	15.5	Yes	3	LSI, AP, A
3	F	8	LSI, U, AG, R	5	17.5	13.5	15	12	7.5	ND	ND	LSI, U, R, EI	ND	Yes	1	LSI, AG, R, EI
4	M	6	LI, U	2	6.5	9	3	12.5	ND	No	15	LI, U, P	ND	No	31	LI, U
5	F	11	U	7.5	22.5	11.5	13.5	7	15.5	Yes	31	LI, R	7.5	No	3	LI, R
6	F	12	LI, U, R	7	3	5	14	3	15.5	Yes	3	LI, U, R	ND	No	15	LSI
7	F	12	LSI, U	4.5	11.5	8	10.5	6.5	15.5	Yes	31	LI, U, AG, R, AP, A	ND	Yes	7	LSI
8	F	12	LSI, U, R	13	3	14	6.5	8	15.5	No	31	LI, AG, R	3.5	ND	ND	LSI
9	M	12	R, NV	4	12.5	8	20	8.5	15.5	No	3	R, NV	15.5	No	3	R, NV
10	M	12	LI, R	3	11	5	12.5	3	15.5	No	31	R, AP, EI	15.5	No	3	LI, R
11	M	8	U	3	3	3	3	3	15.5	ND	ND	U	3.5	ND	ND	U
12	F	9	LSI	0	11.5	4	17.5	9	15.5	No	31	LSI	ND	Yes	ND	LSI
13	M	5	LSI	14	10	13.5	11	11	ND	Yes	ND	LSI	ND	Yes	ND	LSI
14	F	8	LSI, U	3	17.5	8	17.5	11.5	ND	Yes	1	LSI, U	15.5	Yes	ND	LSI, NV, AP, A
15	M	8	LSI	21	35	17	45	24	ND	Yes	ND	LSI	ND	Yes	31	LSI
16	M	9	LSI	0	12.5	4	10	8.5	ND	Yes	1	LSI, TI	ND	Yes	ND	LSI, U
17	M	10	LSI, U, W, R	13.5	10	9.5	9	10	15.5	Yes	15	LSI, U	3.5	Yes	1	LSI, U
18	F	13	LSI, NV	4.5	10.5	3	15	6	15.5	No	1	NV	15.5	Yes	3	LSI, NV, Dy, A
19	M	5	LSI	6.5	15	11	8.5	20.5	ND	Yes	1	LSI, U	ND	Yes	ND	LSI, U
20	F	9	LSI, NV	5.5	14	16.5	17.5	10	15.5	Yes	ND	LSI, NV	15.5	Yes	ND	LSI
21	M	11	LSI, U, R	5	44	21.5	7.5	8.5	ND	Yes	ND	U	15.5	Yes	31	LSI, U
22	M	8	LSI, U, R	12	13.5	6	17.5	9	ND	Yes	ND	LSI, U	ND	Yes	3	LSI, U, R
23	M	10	LSI, U	9.5	11.5	17.5	10	11	15.5	Yes	ND	LSI, U, R	15.5	Yes	ND	LSI, U, NV, R, A
24	F	13	LSI, U	3	2	3	2	5	15.5	No	31	U, W, A	15.5	Yes	ND	LSI, U
25	M	12	W, NV	3	19	12.5	22.5	7.5	3.5	ND	ND	U, AP, LSI, NV, A	ND	Yes	ND	LSI, U
26	M	13	LSI	3.5	13.5	21	8	14	15.5	Yes	ND	LI, U	15.5	No	3	LI, U
27	M	7	LSI	16	15	10	7.5	10.5	3.5	Yes	ND	LSI, U	3.5	Yes	ND	LSI, U
28	M	11	LSI	0	9.5	9	15	15	15.5	Yes	ND	LSI, U	15.5	Yes	ND	LSI, U
29	F	11	LSI, U	2	15.5	17.5	17.5	5	15.5	Yes	ND	LSI, U	15.5	Yes	ND	LSI, U
30	F	6	LSI, U, AG	3	7	9	9.5	5	3.5	Yes	ND	LSI, U, TI	7.5	Yes	ND	LSI, U, AP, A
31	M	8	NV	11.5	10.5	6.5	10	8.5	ND	Yes	ND	LSI, U	ND	Yes	ND	LSI, U, TI
32	F	11	LSI	6.5	5	14	10	10	15.5	Yes	ND	LSI, U	15.5	No	1	LSI, U

A, anaphylaxis; AG, angioedema; AP, abdominal pain; ComSPT, skin prick test using commercial shrimp extract; CP, chest pain; Dy, dyspnea; EI, eye itching; LI, lip itching; LSI, lip swelling/itching; *Mr*, *M. rosenbergii*; ND, not done; NV, nausea/vomiting; P, skin pruritus without rash; *Pm*, *P. monodon*; PTP, prick-to-prick tests; R, rhinitis; SPT, skin prick tests; TI, throat itching; U, urticaria; W, wheezing; F, female; M, male.

Table 4. Demographic data, clinical history, and skin test results, in group IV patients who had negative food challenges to both *P. monodon* and *M. rosenbergii*

No.	Sex	Age (years)	History of reactions to shrimp	Mean weal diameter from skin tests (mm)				
				ComSPT	*Pm*SPT	*Pm*PTP	*Mr*SPT	*Mr*PTP
1	M	6	LSI	8.5	8.5	9	6	7
2	M	14	LI	3	13.5	3	14	9
3	F	11	U	2	6	6.5	4	4.5
4	F	8	U	0	0	0	3.5	5
5	F	13	LSI	2	4	3	4	4
6	M	13	LSI, U, AG	4.5	10	3	3	4
7	F	12	U	10	45	20	5.5	5
8	M	8	LSI, U, W, R	7.5	10	11	15	16

AG, angioedema; ComSPT, skin prick test using commercial shrimp extract; LI, lip itching; LSI, lip swelling/itching; *Mr*, *M. rosenbergii*; *Pm*, *P. monodon*; PTP, prick-to-prick tests; R, rhinitis; SPT, skin prick tests; U, urticaria; W, wheezing.

Table 5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different skin tests

Skin tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ComSPT	88.33	37.50	91.38	30.00
*Pm*SPT	97.73	8.33	66.15	66.67
*Pm*PTP	100	4.17	65.67	100
*Mr*SPT	97.92	5.00	71.21	50.00
*Mr*PTP	100	0	70.59	NA

ComSPT, skin prick test using commercial shrimp extract; *Mr*, *M. rosenbergii*; NPV, negative predictive value; *Pm*, *P. monodon;* PPV, positive predictive value; PTP, prick-to-prick tests; SPT, skin prick test.

(groups II and IV) to *P. monodon* (Fig. 2a). However, only MWD from *Pm*SPT and *Pm*PTP showed statistical significance (median 11.5 vs. 7.5 and 9 vs. 5.25 mm, respectively, $P < 0.05$). Figure 2b shows MWD from different skin tests in patients with positive (groups II and III) and negative challenges (groups I and IV) to *M rosenbergii*. There was no statistical difference between any skin tests in both groups, although MWD from *Mr*SPT were larger in the group with positive than the group with negative challenges to *M. rosenbergii* (median 10.75 vs. 6.75 mm, $P = 0.058$).

We calculated the predictive probability of MWD to determine the outcome of food challenges, and logistic regression was used to establish the reasonable cut-off level (Fig. 3). In all patients, *Pm*SPT provided 80% predictive probability for a positive challenge to *P. monodon* at an MWD of 30 mm. *Pm*PTP and ComSPT provided 95% predictive probability for positive challenges to *P. monodon* at an MWD of 22.5 and 20 mm, respectively (Fig. 3a). *Mr*SPT provided 95% predictive probability for a positive challenge to *M. rosenbergii* at MWD of 30 mm. Predictive probability for a positive challenge to *M. rosenbergii* could not be determined for *Mr*PTP and ComSPT (Fig. 3b).

SPT to *D. pteronyssinus*, *D. farinae,* and *P. americana* extracts were found to be positive in 94.12%, 95.59%, and 80.88% of the patients, respectively.

Discussion

Shrimp is one of the common foods causing mild to life-threatening allergic reactions. It was previously thought that patients with shrimp allergy would develop a hypersensitivity reaction to all shrimps due to the common major allergen, tropomyosin. Species-specific shrimp allergy was reported only in one study, which showed specific shrimp allergens in two shrimp species, white *(Penaeus setifecus*) and brown *(P. aztecus)* shrimp, based on RAST and RAST-inhibition assays [10]. Nevertheless, species-specific shrimp allergy has never been confirmed by a food challenge in the medical literature.

Our study demonstrated that shrimp-allergic children identified by clinical history and skin tests to shrimp could be divided into four groups based on reactions to *P. monodon* and *M. rosenbergii* challenges. Almost half of the patients (47%) developed symptoms to both kinds of shrimp probably due to the cross-reactivity between common allergens. Twelve percent of the patients had no symptoms to both kinds of shrimp. This was supported by the previous study, which showed some discrepancy of self-reported food-induced symptoms and food challenges [14]. Almost 1/5 of the patients developed an isolated allergy to *P. monodon* (23%) or *M. rosenbergii* (18%). Although these two kinds of shrimp are in the same order Decapoda, *P. monodon* is in the suborder Dendrobranchiata and the family Penaeidae, but *M. rosenbergii* is in the suborder Pleocyemata and the family Palaemonidae [15]. The difference in suborder level may explain why these two species may contain unique allergens, that do not cross-react to each other. Further study to identify these unique allergens is ongoing.

Concerning the challenges of raw and cooked shrimp, the proportion of positive challenges to raw shrimp was lower than that of cooked shrimp. The lowest PD of raw shrimp was higher than that of cooked shrimp and none of the patients who ingested raw *M. rosenbergii* developed anaphylaxis. Taken together, the explanations for raw lyophilized shrimp in a capsule causing fewer symptoms than cooked shrimp could be the following. (1) The transit time for the capsule to bypass the oral-mucosal route may result in the reduced symptoms with the raw shrimp as opposed to the more significant reactions with the cooked shrimp. (2) The raw shrimp may be less allergenic than cooked shrimp, and the lyophilization of raw shrimp may denature some of the allergens or the cooking process may

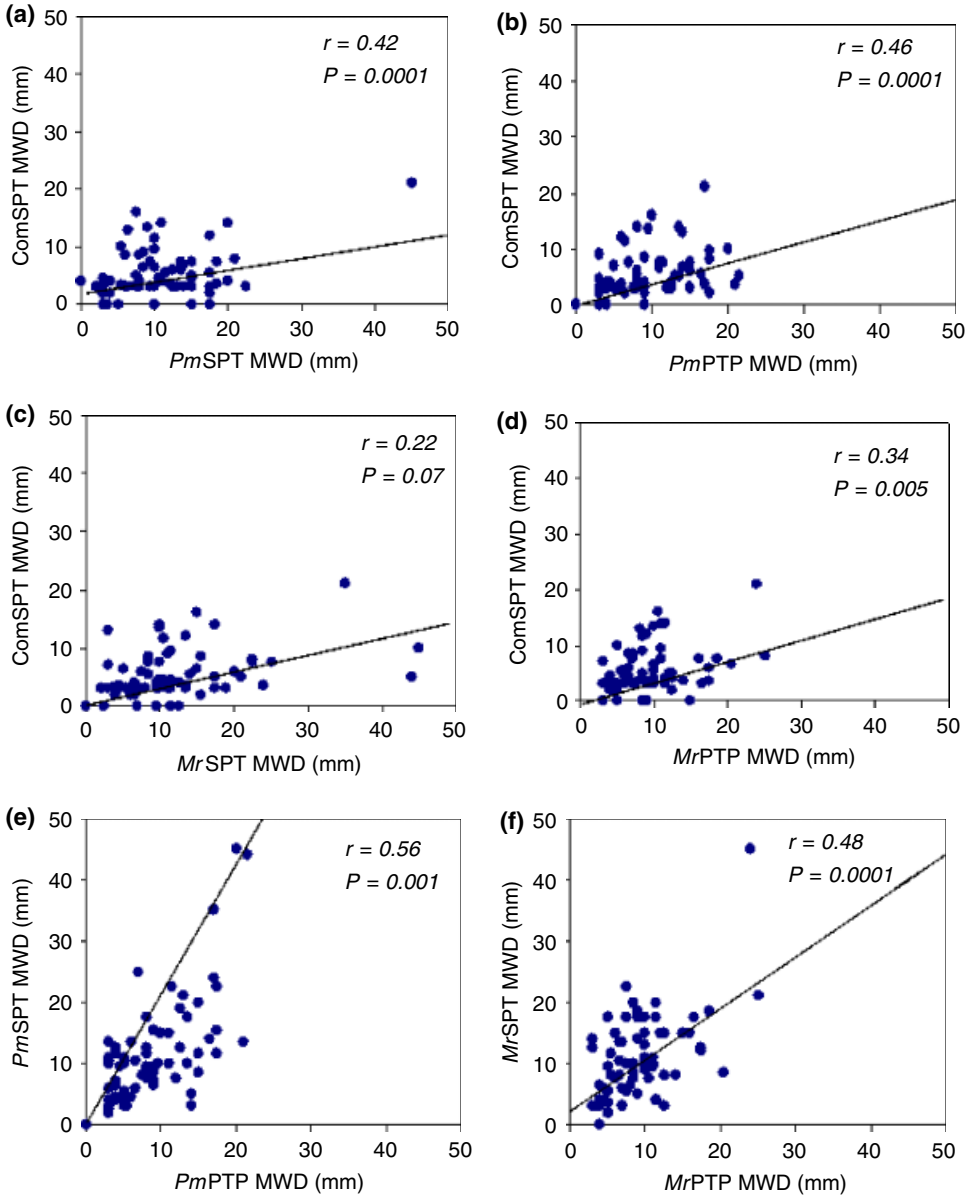

Fig. 1. Correlations between mean weal diameters (MWD) from different skin tests. MWD from different skin tests were tested for correlation coefficient. Dots represent individual values. Regression line, *r*-value for correlation coefficient, and *P*-value are shown.

alter the protein structure or epitope presentation. This hypothesis was supported by the study of Carnes et al that showed that boiled shrimp extract provided higher weal sizes of skin test reactions and higher specific shrimp IgE levels than raw extract in shrimp-allergic patients [16]. (3) The reactions upon challenges with cooked shrimp could be the delayed reactions of raw shrimp challenges. However, there were patients who had positive challenges to cooked *P. monodon* ($n = 7$) and *M. rosenbergii* ($n = 7$) without having raw shrimp challenges. Further study to prove these assumptions is needed.

Our food-challenge protocol contained three steps of challenges with multiple doses in two of the steps. Each step was 15 min apart. The ingestion of capsules bypassed an oral-mucosal reaction. It took two separate days with a 2–4 weeks, rest interval to complete challenges with both species of shrimp. A double-blind, placebo-controlled food challenge was difficult to perform as most patients refused to participate in the study, which would take longer than 2 days. Most reactions were objective symptoms and the frequency of organ involvement correlated well with the clinical history. The most common symptom upon challenges was found in the skin-mucosal system, followed by respiratory, GI, and CVS, which were similar to the results of food challenges to milk, eggs, peanuts, and wheat [17]. Although patients with a history of

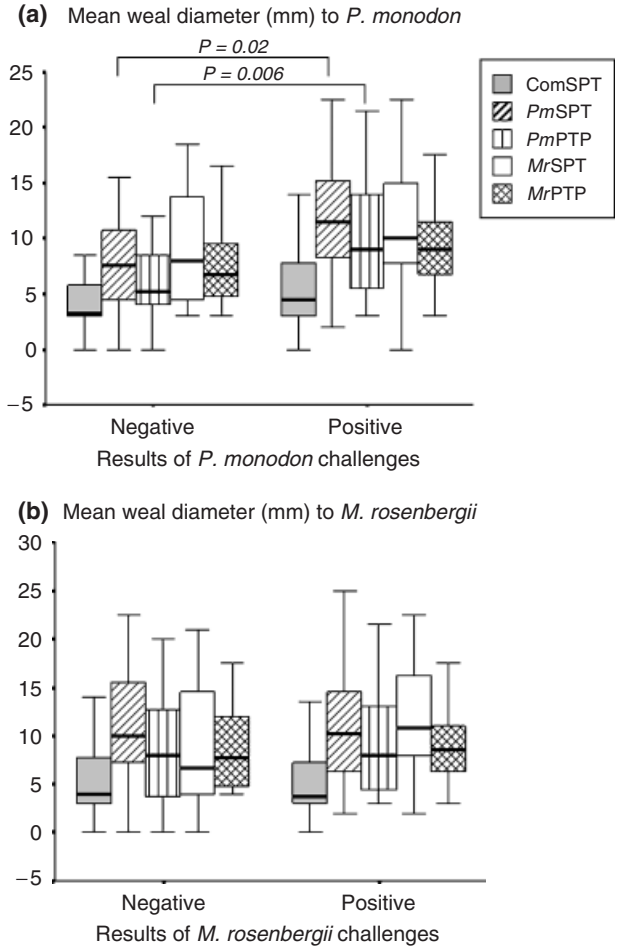

Fig. 2. Mean weal diameter (MWD) from different skin tests in groups of patients with positive or negative challenges to *Penaeus monodon* or *Macrobrachium rosenbergii*. Box plots of MWD from different skin tests in patients with positive (groups I and III) and negative (groups II and IV) challenges to *P. monodon* are shown in (a). (b) Shows MWD from different skin tests in patients with positive (groups II and III) and negative (groups I and IV) challenges to *M. rosenbergii*.

anaphylaxis to shrimp were excluded, our challenges elicited a 12% incidence of anaphylaxis, which responded well to emergency treatment.

All our patients had other underlying allergic diseases with a predominance of respiratory allergy due to the high number of respiratory allergies in our centre. The high rate of sensitization to *D. pteronyssinus* (94%), *D. farinae* (96%), and *P. americana* (81%) in shrimp-allergic children from this study was certainly higher than the sensitization found in children with asthma (44–67%) and allergic rhinitis (37–88%) from our previous reports [18, 19]. This could be explained by the cross-reactivity between tropomyosin in shrimp (group I allergen), house dust mites (Der p 10/Der f 10), and cockroach (Per a 7) or polysensitization in these patients. In the study by Ayuso et al., [7] shrimp-allergic sera recognize 7/8 peptides homologous to Pen a 1 epitopes in Der p 10/Der f 10 and 6/8 epitopes in Per a 7, indicating a high degree of cross-reactivity between tropomyosin from shrimp, house dust mites, and cockroaches.

The comparisons between SPT to commercial and raw shrimp extract or PTP test have never been reported. We observed a moderate correlation between MWD from *Pm*SPT and *Pm*PTP and a fair correlation between MWD from *Mr*SPT and *Mr*PTP. This may extrapolate that SPT using a raw shrimp extract is comparable to the PTP method using cooked shrimp. We demonstrated a fair correlation between MWD from ComSPT–*Pm*SPT, ComSPT–*Pm*PTP, and ComSPT–*Mr*PTP, but not ComSPT–*Mr*SPT. This was not surprising as the commercialized extract was prepared from raw Mexican brown shrimp, *P. aztecus*. Therefore, shrimp in other genus such as *M. rosenbergii* may contain different minor allergens. In general, allergy SPT to food extracts are considered to be sensitive but not specific. The NPV is high, although the PPV is rarely higher than 50% [20]. Our study found that ComSPT had the lowest sensitivity and NPV. This finding was supported by two other reports, which found that the accuracy of SPT using crude extracts (milk, egg, and soy) [21] or the PTP method using fresh foods (fruits and vegetables) [22] was significantly higher than that using commercial food extracts. Besides this, the commercial shrimp extract was made from a different species of shrimp. Perhaps, testing of the appropriate species using a crude extract or a PTP is important. In the cases of shrimp allergy, the NPV of 30% from ComSPT may be unacceptable because a significant number of sensitive patients could have been missed. This is considered to be dangerous in cases of anaphylaxis if patients are allowed to take shrimp without further challenge.

Figure 2a shows a significantly larger MWD from *Pm*SPT and *Pm*PTP but not ComSPT in patients with positive as opposed to negative challenges to *P. monodon*. In contrast, MWD from all the five skin tests could not show statistical difference between the patients with positive and negative challenges to *M. rosenbergii* (Fig. 2b). However, MWD from *Mr*SPT showed a trend to be larger in the patients with positive than negative challenges to *M. rosenbergii*.

SPT to food is a useful tool for identification of food sensitization. However, confirmation of food allergy requires oral food challenges. In practice, some allergy centres consider food challenges to be time consuming, expensive, and a potential cause for serious outcome including life-threatening anaphylaxis. Therefore, a recent study has attempted to identify the weal size from SPT to specific foods that predicted the outcome of oral food challenges [23]. In this study, weal sizes of SPTs to cow's milk and hen's egg were identified to predict probabilities of positive food challenges. In our study, predictive probabilities of SPT to shrimp were calculated to determine positive challenges in

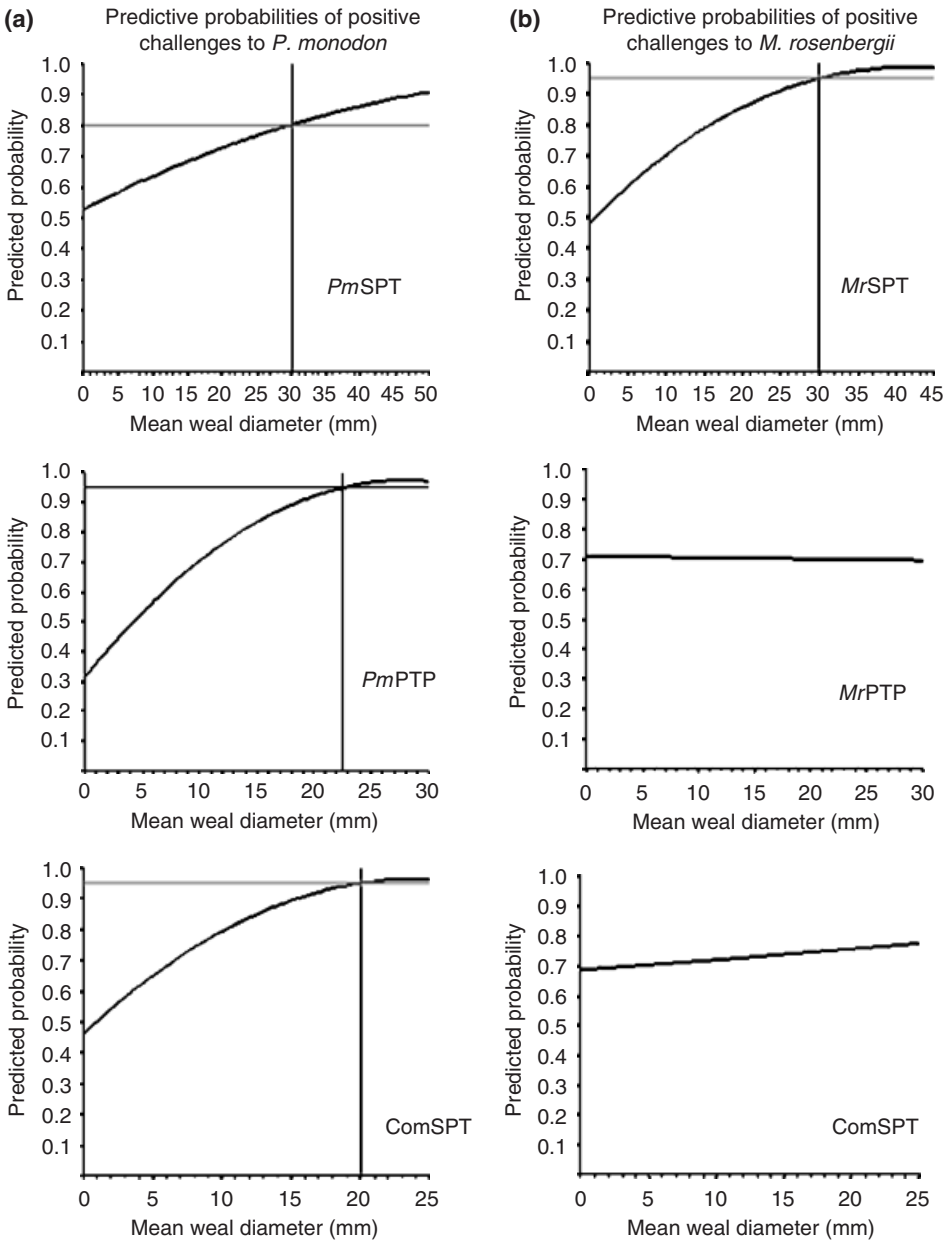

Fig. 3. Predictive probabilities for mean weal diameter (MWD) from different skin tests to determine the positive food challenges. Predictive probabilities for MWD (mm) from different skin tests to determine positive food challenges are shown. (a) and (b) Demonstrate predictive probabilities of skin tests for a positive challenge to *Penaeus monodon* and *Macrobrachium rosenbergii*, respectively. Thin lines represent predicted probability and MWD.

patients with shrimp allergy. These cut-off levels may be useful in the clinical setting where a food challenge test is not feasible. However, a large study population with multiple ethnicities may be needed to set a standard cut-off value.

In conclusion, we demonstrated patients with a specific allergy to *P. monodon* or *M. rosenbergii* confirmed by food challenges. The correlations of SPT using raw shrimp extracts and PTP using cooked shrimp of both species as well as commercial shrimp extract were studied. The importance of these skin tests to differentiate between the groups with positive and negative challenges as well as the predictive probability of MWD to identify positive challenge was determined.

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